Myeloid Leukemia Factor Acts in a Chaperone Complex to Regulate Transcription Factor Stability and Gene Expression

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http://dx.doi.org/10.1016/j.jmb.2016.10.026
Edited by Bienz Mariann

Abstract

Mutations that affect myelodysplasia/myeloid leukemia factor (MLF) proteins are associated with leukemia and several other cancers. However, with no strong homology to other proteins of known function, the role of MLF proteins in the cell has remained elusive. Here, we describe a proteomics approach that identifies MLF as a member of a nuclear chaperone complex containing a DnaJ protein, BCL2-associated anthanogene 2, and Hsc70. This complex associates with chromatin and regulates the expression of target genes. The MLF complex is bound to sites of nucleosome depletion and sites containing active chromatin marks (e.g., H3K4me3 and H3K4me1). Hence, MLF binding is enriched at promoters and enhancers. Additionally, the MLF-chaperone complex functions to regulate transcription factor stability, including the RUNX transcription factor involved in hematopoiesis. Although Hsc70 and other co-chaperones have been shown to play a role in nuclear translocation of a variety of proteins including transcription factors, our findings suggest that MLF and the associated co-chaperones play a direct role in modulating gene transcription.

Introduction

Development of the hematopoietic system is a well-regulated process involving specific factors that determine cell fate and lineage specification. The myelodysplasia/myeloid leukemia factors (MLFs) are a poorly characterized family of proteins involved in hematopoiesis that associate with chromatin and may regulate transcription. The founding member of the MLF family is human MLF1, which was originally identified in a fusion protein with nucleophosmin (NPM) and is associated with myelodysplastic syndrome and acute myeloid leukemia [1]. The resulting NPM–MLF1 fusion contains the NPM oligomerization domain and nuclear localization signals, causing it to accumulate in the nucleus. Although fusion with NPM causes other proteins to become oncogenic, hMLF1 itself is also implicated in cancer: elevated levels of endogenous hMLF1 are observed with high frequency in myeloid cell malignancies, and this increased expression corresponds with poor prognosis and low survival rates [2–4]. In addition to the misregulation of hMLF1 in leukemia, MLF proteins function in normal hematopoiesis. hMLF1 expression is observed in early hematopoietic CD34+ progenitor cells, and its expression levels decrease during differentiation [2,5]. Murine MLF1 suppresses erythroid differentiation, while its overexpression promotes myeloid maturation [5]. The suppression of erythroid differentiation by MLF1 is mediated through the inhibition of proteasomal

degradation of $p27^{Kip1}$, resulting in the inhibition of cyclin-E/cdk2 complexes [6]. Although hMLF1 is most well characterized in hematopoietic cells, human and mouse MLF1 are normally expressed in a variety of adult and fetal tissues, with the highest levels seen in the testis, skeletal muscle, and heart [1,7]. However, the role of MLF proteins in these tissues is not understood, and its mechanism of action in hematopoietic cells remains unclear.

MLF is highly conserved in metazoans, and mammals contain two paralogs, MLF1 and MLF2, with approximately 40% identity [8,9]. *Drosophila melanogaster* contains a single MLF ortholog, dMLF that shares equal homology with hMLF1 and hMLF2 [8]. Thus, *Drosophila* provides a genetically tractable model system in which we can examine the mechanism of MLF function. As observed for hMLF1, the subcellular localization of dMLF is dependent on cell type, and its expression levels change during development [8]. Additional studies in *Drosophila* demonstrated that dMLF associates with chromatin, suggesting a nuclear role for MLF proteins [10]. Likewise, hMLF1 associates with DNA, and exogenous expression of hMLF1 regulates the transcription of several genes involved in differentiation and cell growth [11]. Importantly, null alleles of *mlf* in *Drosophila* are embryonic lethal, indicating that it plays an important role during development [8]. Although studies have reported that both human and *Drosophila* MLF proteins are in the nucleus and have suggested roles for this localization, little is known about their specific function within the nuclear compartment or how MLF proteins direct cell differentiation.

Another group of proteins whose nuclear functions are less understood are molecular chaperones. Although the functions of molecular chaperones in protein folding have been extensively studied, other functions for these proteins are starting to emerge, and these include roles in endocytosis, nucleocytoplasmic shuttling, signal transduction, and multiprotein complex assembly [12]. Of particular interest is the recently described role for molecular chaperones at chromatin [13,14]. Most studies examining the involvement of molecular chaperones in transcriptional regulation have focused on their functions in protein folding and stabilization. However, a few recent studies have suggested that molecular chaperones might play a direct role at chromatin in regulating gene expression. For example, studies in yeast have revealed that both Hsp90 and Hsp70 systems are required at chromatin for proper removal of promoter-bound nucleosomes to allow for gene induction [13]. Additionally, studies in *Drosophila* found that Hsp90 regulates gene expression by localizing to transcription start sites (TSS) of genes that display RNA polymerase II (Pol II) promoter-proximal pausing via stabilization of the negative elongation factor (NELF) complex, and therefore, it paused Pol II [14]. Thus, molecular chaperones appear to play a broader role in the cell than previously thought, regulating both post-translational events and transcription. These Hsp70 and Hsp90 studies in yeast and *Drosophila* raise the question of whether other molecular chaperones might also play direct roles in transcription, and it prompted us to explore heat shock protein 70 (Hsc70) when it co-purified with MLF (below).

The Hsp70 family of co-chaperones, a major class of these proteins, is conserved from bacteria to humans [12]. The well-characterized Hsc70 protein is constitutively expressed in all organisms. It contains an amino-terminal ATP-binding domain and a carboxy-terminal substrate-binding domain. Through the concerted actions of the ATPase activity of the ATP-binding domain and the substrate-binding domain, Hsc70 binds to and folds both native and unfolded client proteins. The intrinsic ATPase activity of Hsc70 is rather weak and is stimulated by binding to co-chaperone proteins [15]. The ATP-binding domain of Hsc70 interacts with the Hsp40/DnaJ co-chaperones through a conserved J domain [15]. The binding of DnaJ proteins to Hsc70 enhances the ATPase activity of Hsc70, facilitating the hydrolysis of ATP to ADP, which causes a conformational change in the substrate-binding domain of Hsc70. This conformational change results in high-affinity binding of Hsc70 to the unfolded client protein, thereby enabling its folding. Nucleotide exchange factors (NEFs) are co-chaperones that then stimulate the release of the client protein by facilitating the exchange of ADP for ATP, thus resetting the Hsc70 folding cycle [16]. One class of NEFs that interact with Hsc70 is the BAG family of proteins, which contain a conserved BAG domain that binds to the ATP-binding domain of Hsc70. In addition to stimulating the activities of Hsc70, co-chaperone proteins are thought to add functional specificity to the Hsc70 chaperones, allowing the small family of highly conserved Hsc70 proteins to perform a large variety of specific cellular functions [15].

To examine the functions of MLF proteins in the nucleus, we employ a proteomics approach to identify MLF-interacting proteins in nuclear extracts. Interestingly, we observed that *Drosophila* MLF interacts with the co-chaperones DnaJ-1 and BCL2-associated anthanogene 2 (BAG2) in the nucleus, along with the molecular chaperone Hsc70–4. Our observations indicate that members of the MLF-chaperone complex co-localize to the same sets of genomic loci, suggesting that this complex plays a broader role in the nucleus than simply folding or shuttling proteins into this compartment. Specifically, MLF and DnaJ-1 associate with regions of open chromatin at promoters marked by the histone modification H3K4me3 and at active enhancers marked by H3K27ac and H3K4me1. Additionally, we find that a variety of transcription factors co-purify with the MLF-chaperone complex using size-exclusion chromatography. Notably, we identified a change in the stability of these transcription factors, both activators and repressors, in the